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Nonmyeloablative Bone Marrow Transplantation: Infectious Complications in 65 Recipients of HLA-Identical and Mismatched Transplants

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ABSTRACT

Infections are a common complication of allogeneic bone marrow transplantation and the leading cause of transplantation-related mortality. It had been hypothesized that transplantation following nonmyeloablative preparative regimens would result in fewer infections by causing less mucosal injury, less graft-versus-host disease, and allowing earlier immune reconstitution. We have retrospectively reviewed the infectious complications of 65 consecutive patients with advanced hematologic malignancies who underwent bone marrow transplantation using a novel preparative regimen consisting of cyclophosphamide, thymic irradiation, and in vivo T-cell depletion. Cytomegalovirus (CMV) infection occurred in 52% of cases in which the donor or recipient had evidence of prior CMV exposure. Using a strategy of preemptive therapy and secondary prophylaxis with ganciclovir, no CMV disease occurred. Infections with gram-positive bacteria predominated over the first 100 days after bone marrow transplantation. Thereafter, the relative proportion of gram-negative infections increased without a significant increase in episodes of neutropenia. The rate of bacterial infections was not influenced by relapse of the underlying malignancy. Seven patients developed infections with *Aspergillus* species, which was the most common infectious cause of death in these patients. Infections with viruses other than CMV (n=10) and with protozoan organisms (n=2) also occurred. The use of HLA-mismatched donors, the occurrence of grade II-IV acute graft-versus-host disease, and treatment with corticosteroids did not influence the risk of CMV or bacterial or fungal infections in patients who underwent transplantation following this preparative regimen. Overall, the incidence and spectrum of infections in this series was similar to the reported incidence of infections following conventional myeloablative allogeneic stem cell transplantation. We conclude that a quantitative T-cell deficiency in these extensively T-cell depleted patients may be a risk factor for infection, even in the absence of graft-versus-host disease.

KEY WORDS

Bone marrow transplantation • Complications • Nonmyeloablative • Infection • Cytomegalovirus • Fungal infection

INTRODUCTION

Despite major advances in supportive care, infections almost invariably occur after an allogeneic bone marrow transplantation and remain a cause of significant morbidity and mortality [1]. Infections are reported to account for up to 63% of deaths in allogeneic transplantation recipients at the time of autopsy [2]. Susceptibility to infection is closely linked to the degree of donor and host histocompatibility, graft-

versus-host disease (GVHD), and immunosuppression. Although infections with common bacterial organisms occur in this setting, transplantation recipients are uniquely susceptible to infection with atypical or opportunistic pathogens such as cytomegalovirus, fungi, and atypical mycobacteria. Numerous studies designed to define the role of antimicrobial prophylaxis in this patient population have been carried out, and extensive guidelines based on these studies have recently been adopted [3].

Nonmyeloablative stem cell transplantations have been carried out in a number of centers for patients with advanced or refractory hematologic malignancies [4-6]. The potential for reduced toxicity with these nonmyeloablative regimens makes them an attractive treatment option in this frequently debilitated group of patients. Furthermore, a reduced duration of neutropenia, earlier withdrawal of immunosuppression, and the potential for earlier and more complete immune reconstitution could result in fewer posttransplantation infectious complications.

At our center, nonmyeloablative stem cell transplantations have been carried out using a novel preparative regimen consisting of high-dose cyclophosphamide, thymic irradiation, and in vivo T-cell depletion using antithymocyte globulin or anti-CD2 monoclonal antibody therapy followed by HLA-matched or haploidentical donor bone marrow transplantation. Prophylactic donor lymphocyte infusions are administered to patients without GVHD approximately 35 days posttransplantation, after resolution of regimen-related tissue injury and establishment of mixed donor-host lymphoid chimerism, in an effort to induce a more potent immune-mediated antitumor response. This regimen has been previously reported to reliably induce mixed chimerism after HLA-matched or partially mismatched donor stem cell transplantation, often with significant antitumor responses among patients with advanced hematologic malignancies [7,8].

Given the potential for reduced toxicity and improved immunocompetence of recipients of nonmyeloablative stem cell transplantations, we undertook a review of the infectious complications in 65 recipients of these transplants in our center.

PATIENTS AND METHODS

Patients

Sixty-five patients with advanced hematologic malignancies received a nonmyeloablative bone marrow transplantation between April 1997 and December 2000. These patients were analyzed for infectious complications through May 2001. All patients underwent transplantation on protocols approved by the Massachusetts General Hospital (Boston, MA) Subcommittee for Human Studies, and all subjects gave written informed consent. Eligibility criteria included a diagnosis of advanced hematologic malignancy, physiologic age $\leq$70, Eastern Cooperative Oncology Group performance status of 0, 1, or 2, and adequate organ function. Patients and donors were HLA typed using serologic methods for class I antigens and high-resolution polymerase chain reaction-based methods for HLA-DR. Before transplantation, donors and recipients were screened for serologic evidence of exposure to *Toxoplasma gondii*, cytomegalovirus (CMV), herpes simplex virus 1 and 2, hepatitis B and C, human T-lymphotrophic virus 1 and 2, and the human immunodeficiency virus types 1 and 2.

Conditioning Therapy

Conditioning chemotherapy consisted of cyclophosphamide 50 mg/kg/day for 3 (n=53) or 4 (n=12) days before transplantation, from day -6 or -5 to day -3. Thymic irradiation was given at a dose of 700 cGy on day -1 to all patients who had not had prior mediastinal radiotherapy (n=44). T-cell depleting antibodies were used in all cases. Antithymocyte globulin (ATG; ATGAM, Pharmacia-Upjohn, Kalamazoo, MI) was initially given at a dose of 30 mg/kg on days -2, -1 and $+1$ (n=8). Because of toxicity, the dose was reduced to 15 mg/kg/day on days -1, $+1$, $+3$, and $+5$ (n=27). The ATG dose was subsequently changed to 20 mg/kg/day on days -1, $+1$, $+3$, and $+5$ (n=22). MEDI-507 (BioTransplant Inc, Charlestown, MA), a humanized anti-CD2 monoclonal antibody, was given to 8 recipients of HLA 2- or 3- antigen mismatched transplants. Four patients received a test dose of MEDI-507 0.1 mg/kg on day -2, followed by 0.6 mg/kg on days -1, 0, and $+1$. Four patients received a test dose of MEDI-507 0.1 mg/kg on day -7 and 0.6 mg/kg doses on days -6 and -5. Bone marrow was collected as described on day 0 and infused over 1 to 2 hours [7].

Graft-versus-Host Disease Prophylaxis and Treatment

Cyclosporine 5 mg/kg/day was started by continuous infusion on day -1. The dose was reduced on day $+4$ to 3 mg/kg/day and adjusted to maintain whole blood levels within the therapeutic range (200-300 ng/mL). Dosage adjustments were made for significant renal dysfunction. Cyclosporine (Sandimmune; Novartis Pharmaceuticals Corp, East Hanover, NJ) was given twice daily by mouth at a starting dose of 12 mg/kg/day once oral medications were tolerated. In the absence of acute GVHD, cyclosporine was tapered and discontinued by approximately day $+35$ in preparation for a donor lymphocyte infusion.

Engraftment syndrome [9] and acute GVHD were treated with intravenous methylprednisolone 1 to 2

mg/kg/day in 2 divided doses for recipients of HLA-matched bone marrow stem cells. A starting dose of intravenous methylprednisolone of 2 to 10 mg/kg/day was used in recipients of partially mismatched bone marrow grafts.

Donor Leukocyte Infusion

Donor peripheral blood mononuclear cells were collected by apheresis on or before day +35. An initial infusion on or after day +35 consisting of 1×10^7/kg $CD3^+$ cells was given to 20 transplant recipients without evidence of acute GVHD for conversion from mixed chimerism to full donor chimerism. A second infusion of 1 to 5×10^7/kg $CD3^+$ cells was given on day 56 per protocol to 3 patients. The protocol was subsequently amended to allow a second infusion of donor lymphocytes only for patients without acute GVHD in whom donor chimerism was less than 90% at 4 weeks after the first infusion.

Antimicrobial Prophylaxis

Surveillance cultures of blood, stool, urine, and oropharynx were taken at the time of admission and then weekly until discharge. Patients used chlorhexidine mouthwash for mouth care and showered with chlorhexidine daily.

On admission, patients were started on co-trimoxazole and an oral fluoroquinolone daily for prevention of *Pneumocystis carinii* and bacterial infections, respectively. Cotrimoxazole was continued until day −1 and was then given 3 times weekly from the time of engraftment. Patients unable to take cotrimoxazole received intravenous pentamidine 5 mg/kg monthly or oral atovaquone. Oral fluconazole 400 mg was given on day −1, and then reduced to 200 mg orally daily. Acyclovir 250 mg/m^2 every 8 hours intravenously was started on day −1 and then changed to the oral route of administration after engraftment had been achieved. *P carinii* prophylaxis, fluconazole and acyclovir, were continued until patients showed no further evidence of GVHD.

Supportive Care

An indwelling double-lumen silastic central venous catheter was placed before transplantation. Dressings were changed 3 times weekly and the catheter site was examined for signs of infection on a daily basis. Topical antibiotic ointment was applied to the site with each dressing change. All patients were cared for in high-efficiency particulate air filtered or laminar airflow rooms. Reverse isolation was continued until the time of neutrophil engraftment, defined as the first day of absolute neutrophil count >500/μL. Fever higher than 100.5° F in neutropenic patients was treated empirically with broad-spectrum antibiotics, usually ceftazidime and vancomycin, and antibiotics were adjusted based on microbial sensitivity testing for patients with positive cultures. Antibiotics in febrile neutropenic patients were continued until the time of engraftment.

Cases in which both donor and recipient were negative for CMV antibodies received blood that was serologically negative for CMV. All transplantation recipients were monitored weekly using a CMV pp65 antigenemia assay. Two slides were examined and patients with 1 or more positive cells were treated with ganciclovir as follows. Induction consisted of ganciclovir 5 mg/kg intravenously 2 times daily for 10 days to 2 weeks followed by ganciclovir 5 mg/kg intravenously daily until 2 consecutive weekly negative CMV pp65 antigenemia assays were obtained. Patients were maintained on oral ganciclovir 1000 mg twice daily for at least the first 6 months posttransplantation or until they no longer required treatment with immunosuppressive medications. Doses were adjusted clinically according to renal function and blood counts. Patients who experienced myelosuppression while on ganciclovir could be treated with granulocyte colony-stimulating factor (Neupogen; Amgen Thousand Oaks, CA) as clinically indicated. In cases where CMV antigenemia failed to clear in a timely manner or when patients were unable to tolerate ganciclovir, anti-CMV hyperimmune globulin could be administered.

Intravenous immunoglobulin 0.5 g/kg was given monthly to patients with IgG levels of <400 mg/dL.

Statistical Analysis

Data on infection, relapse, and mortality were collected prospectively for use in this analysis. An infectious episode was defined on the basis of positive culture or antigen tests. Positive bacterial or fungal cultures from normally sterile sites were considered to represent an infection, while cultures from nonsterile sites were considered to represent an infection only in the presence of corroborative clinical signs. Two cultures positive for the same organism obtained from the same site within a 2-week period were considered to represent a single infection. *Aspergillus* and *Nocardia* were exceptions to this: 2 cultures positive for either of these organisms were considered to represent the same infection, regardless of the time or site of sampling. The day of onset of acute GVHD was taken as the day on which the first biopsy consistent with this diagnosis was sent. In 3 cases, a diagnosis of grade II-IV acute GVHD was made without histologic confirmation. In these cases, the date of onset is taken as the day on which treatment for acute GVHD was instituted. Infectious episodes for patients who experienced engraftment failure, as indicated by a return to fully recipient bone marrow chimerism, were not censored, although these patients were no longer considered to be at risk for GVHD. The actuarial rates of

Table 1. *Patient Characteristics at Time of Transplantation*

	HLA Matched	HLA Mismatched
No. of Patients	43	22
Median age (range)	44 (22–62)	35 (16–58)
Percent male	60%	64%
Diagnosis		
NHL	26 (60%)	17 (77%)
HL	6 (14%)	1 (4.5%)
AML	5 (12%)	2 (9%)
ALL	1 (2%)	
CLL	4 (9%)	1 (4.5%)
MM	1 (2%)	1 (4.5%)
Prior autologous transplant	13 (30%)	1 (4.5%)

Abbreviations NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; MM, multiple myeloma.

GVHD onset, first CMV infection, and first bacterial infection were estimated by the Kaplan-Meier method. Their associations with HLA matching and CMV serostatus were analyzed by the log-rank test, while the effect of GVHD was modeled as a time-varying covariate in a proportional hazards regression. Poisson regression was used to examine the relationship between the incidence rates of CMV infection, bacterial infection, and *Aspergillus* infection with the onset of GVHD, initiation of steroids, or relapse. In the analysis of the effect of GVHD on the incidence of infection, the follow-up time and infectious episodes before the onset of GVHD and after loss of chimerism contribute to the risk in the no-GVHD group. This approach is analogous to the treatment of GVHD as a time-dependent covariate by Osarogiagbon et al. [10], while allowing for multiple infectious episodes per patient.

RESULTS

Sixty-five patients underwent nonmyeloablative bone marrow transplantation between April 1997 and December 2000. Pretransplantation characteristics are shown in Table 1. In the case of HLA-mismatched transplants, donors were mismatched for 1 (n=5), 2 (n=15), or 3 (n=2) HLA antigens in the GVHD direction. Patients were extensively pretreated before undergoing transplantation. Median number of prior chemotherapy regimens was 3 (range, 0-8) and 22% had prior autologous stem cell transplantations. At the time of transplantation 64 of 65 patients had chemotherapy-refractory disease or had received a prior autologous stem cell transplantation. One patient was in partial remission following salvage chemotherapy for non-Hodgkin's lymphoma. Median time from diagnosis was 19.2 months (range, 5-115.8 months) for recipients of fully matched bone marrow transplants and 18.7 months (range, 4.7-185 months) for recipients of mismatched bone marrow.

One year after transplantation, the cumulative incidence of grade II-IV acute GVHD or de novo extensive chronic GVHD was 55%. Median actuarial time to onset of GVHD was 188 days. Graft rejection occurred in 19 of 65 patients (29%) a median of 44 days (range, 20-143 days) after transplantation. Autologous reconstitution occurred promptly in all patients who experienced graft rejection. Thirty-nine patients (60%) showed evidence of disease progression a median of 119 days (range, 0-908 days) posttransplantation.

Cytomegalovirus

Twenty-nine of 43 recipients (67%) of matched bone marrow grafts were at risk for CMV infection while 13 of 22 recipients (59%) of mismatched grafts were at risk. Overall, 52% of patients at risk experienced reactivation of latent CMV infection. Eighty-two percent of patients who reactivated CMV did so for the first time in the first 60 days. All patients who reactivated did so for the first time within 100 days of transplantation. Cytomegalovirus infection occurred a median of 38.5 days (range, 11-98 days) after transplantation in recipients of HLA-matched bone marrow and after a median of 25 days (range, 21-87 days) in recipients of HLA-mismatched bone marrow (P=.823). Other pretransplantation variables, such as cyclophosphamide dose (P=.218), use of thymic irradiation (P=.141), use of ATG or MEDI-507 for in vivo T-cell depletion (P=.672), or use of therapeutic donor lymphocyte infusion (DLI) (P=.807), did not influence the development of CMV infection. Patients who received prophylactic DLI were twice as likely to develop CMV infection after transplantation (P=.021).

Nine patients (41%) required treatment for a second episode of CMV infection at a median of 122 days (range, 56-392 days) after transplantation. Four patients were antigenemic on a third and fourth occasion between 165 and 351 days and 223 and 483 days, respectively. Recipients of mismatched transplants cleared CMV antigenemias more slowly than did recipients of HLA-matched transplants, at a median of 14 days (range, 1-86 days) versus 5 days (range, 1-18 days) (P=.0497). The peak number of white blood cells positive for CMV pp65 antigen was 3 (range, 1-10) for recipients with HLA-matched donors, versus 9 cells (range, 1-274) for those with mismatched donors (P=.117).

Donor and recipient serostatus was a strong predictor of CMV infection posttransplantation (Figure 1). The highest incidence of CMV infection occurred in cases in which both donor and recipient were serologically positive for CMV antibodies. In this situa-

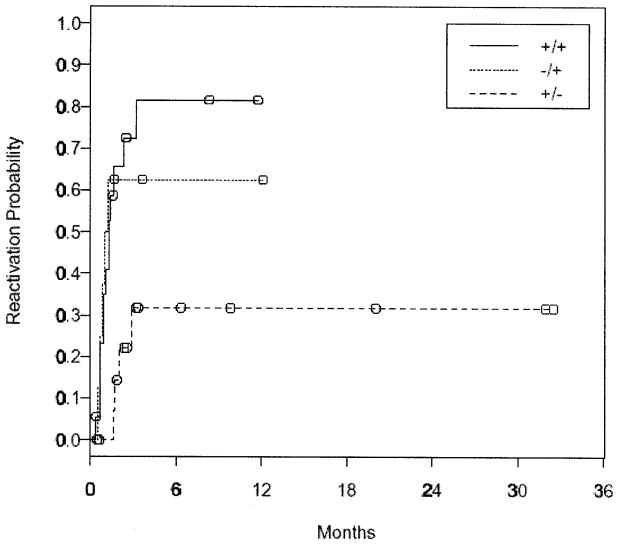

Figure 1. First CMV infection of exposed patients by donor/recipient serostatus. Kaplan-Meier product-limit estimates of the actuarial risk of experiencing a first episode of CMV viremia for cases in which donor and recipient are both positive (+/+, actuarial risk 82%), donor is negative and recipient is positive (-/+, actuarial risk 63%), or donor is positive and recipient is negative (+/-, actuarial risk 32%). Cases in which neither donor nor recipient was positive are not indicated because no such patients were found to have CMV viremia.

tion, 82% of recipients became viremic at a median of 40 days (range, 11-98 days) posttransplantation. This compares with 43% of patients experiencing infection if only 1 member of the donor–recipient pair was positive for CMV antibodies (P=.015). Cytomegalovirus infection was less likely if the recipient was CMV negative. Infection occurred in 32% of CMV-negative recipients who received transplants from CMV positive donors, compared with 76% of CMV-positive recipients who received transplants from donors who were either CMV positive or negative (P=.002). The rate of infection of seropositive recipients was not affected by the serostatus of the donor (P=.827). Median time to infection was significantly shorter when both donor and recipient were positive for CMV as compared with when only the donor was CMV positive (40 days [range, 11-98 days] *v* unreached with a plateau of 32% of patients reactivating after 87 days [range, 50-87 days]; P=.002). No CMV-negative patient receiving a transplant from a CMV-seronegative donor became viremic following transplantation.

Cytomegalovirus reactivated 24 times over 7002 patient-days of follow-up of patients without GVHD and 15 times over 6874 patient-days of follow-up of patients with GVHD (P=.107). There were 28 episodes of viremia in at-risk patients treated with steroids over 9342 patient-days of follow-up, compared with 11 episodes of viremia over 4534 patient-days of follow-up among patients not receiving treatment with steroids (P=.552).

Twenty-two patients received treatment for CMV infection. Two of these cases were intermittently lost to follow-up, leaving insufficient information to evaluate the tolerability of CMV treatment. During the induction phase of ganciclovir therapy, 3 patients developed neutropenia and required treatment with granulocyte colony-stimulating factor. In no case was ganciclovir dosing reduced during induction because of myelosuppression. Two patients were treated with CMV hyperimmune globulin in addition to ganciclovir during induction because of delayed clearance of CMV antigen. Foscarnet was added to ganciclovir in 1 of these cases, although viral sensitivity testing subsequently confirmed sensitivity to nucleoside analogues. Three patients died during ganciclovir induction, 2 from progression of their malignancies on days +40 and +41 and 1 from disseminated aspergillosis on day +107. Fourteen patients received maintenance therapy with oral ganciclovir. Information on the course of maintenance therapy was unavailable in 2 patients known to have received it. Dosage adjustment was required for myelosuppression in 6 of the 12 remaining patients. Three patients required granulocyte colony-stimulating factor, and maintenance therapy had to be stopped in 2 cases. One patient received treatment with CMV hyperimmune globulin for maintenance in addition to reduced-dose ganciclovir. Three patients became viremic while on maintenance ganciclovir, and in all cases viremia resolved with increased doses of oral ganciclovir. In all cases of breakthrough viremia the ganciclovir dose had been reduced because of myelosuppression. Cytomegalovirus disease did not occur in any of these patients.

Bacterial Infections

Overall, 87 cultures were positive for bacterial organisms in 79 separate infections. These included 53 infections with gram-positive organisms alone and 25 infections with only gram-negative organisms. One patient had a mixed infection with both a gram-positive and a gram-negative organism (Table 2). Coagulase negative *Staphylococcus* was the most commonly identified organism, causing 33 infections. These include 26 bacteremias, which occurred at a median of 64 days (range, 3-383 days) posttransplantation. Two wound swabs became positive for coagulase negative *Staphylococcus* and 1 patient was diagnosed with osteomyelitis and secondary septic arthritis caused by this organism on day +71. The latter patient was also bacteremic with coagulase-negative *Staphylococcus* on day +111. Central venous catheter tip cultures were positive for coagulase-negative *Staphylococcus* on 4 occasions.

The spectrum of bacterial infections in the posttransplantation period is shown in Table 2. The risk of developing bacterial infection remained present well

Table 2. *Causes of Bacterial Infections in Recipients of Nonmyeloablative Stem Cell Transplants*

	Days Posttransplant			
	0–30	31–60	61–100	>100
Evaluable patients (n)	65	61	54	44
No. of patients with bacterial infections (%)	12 (18%)	13 (21%)	11 (20%)	21 (48%)
No. of bacterial infections	14	14	13	38
Gram-positive Organisms (n)	12 CNS (6) Streptococcus mitis (2) Enterococcus (1) Clostridium difficile colitis (2) Clostridium innoccuum (1)	10 CNS (9) Corynebacterium and Peptostreptococcus (1)	11 CNS (8) S aureus (2) Bacillus and CNS (1)	20 CNS (10) S aureus (2) Enterococcus (2) Strep. bovis (1) Strep. Pneumoniae (1) Nocardia (3) Listeria monocytogenes (1)
Gram-negative Organisms (n)	2 Pseudomonas (1) Roseomonas (1)	4 Klebsiella (1) Pseudomonas (1) Campylobacter (1) Xanthomonas and Klebsiella (1)	2 Campylobacter (1) Pseudomonas (1)	18 Pseudomonas (5) Acinetobacter (1) Klebsiella (2) Serratia (1) E coli (1) Bacteroides (2) Campylobacter (2) Hemophilus influenzae (2) Pseudomonas and Klebsiella (1) Acinetobacter, Pseudomonas and Enterococcus (1)

Abbreviation: CNS, coagulase-negative staphylococcus.

after 100 days posttransplantation, and infections with low virulence organisms occurred well into the second year. For example, 5 cultures from 3 patients showed *Nocardia nova* species at a median of 189 days (range 165-977 days) posttransplantation. Two of these were diagnosed on blood culture, 1 on sputum culture, and 1 on culture of pleural fluid in a patient with a large pleural effusion. One of these patients was subsequently found to also have meningitis with *Nocardia*. A recipient of a mismatched transplant presented with headache and mental status changes on day +427. A culture of cerebrospinal fluid was positive for *Listeria monocytogenes*. Among patients surviving for more than 100 days, the relative proportion of gram-negative infections increased dramatically, accounting for 47% of infections during this period. However, neutropenia was present in only 2 patients with gram-negative infections beyond day 100.

The sites of bacterial infection are shown in Table 3. While bacteremia remained common even late posttransplantation, the relative frequency declined with time. An increase in the frequency of nonbacteremic bacterial infections, particularly involving the lung, skin, and soft tissues, can be appreciated.

The time to first bacterial infection was not affected by the degree of HLA matching between donor and recipient and was 109 days (range, 3-836 days) for the recipients of matched stem cells and 75 days (range, 3-192 days) for the recipients of mismatched transplants (P=.403). Other pretransplantation variables, such as cyclophosphamide dose (P=.997), use of MEDI-507 or ATG for T-cell depletion (P=.248), and the use of prophylactic DLI (P=.456) did not influence the rate of bacterial infections. There was a 49% increase in bacterial infections among patients who were given thymic irradiation before undergoing transplantation, although this association was not sta-

Table 3. *Sites of Bacterial Infections in 65 Recipients of Nonmyeloablative Bone Marrow Transplants*

	Days Posttransplant			
	0–30	31–60	61–100	>100
Bacteremia	11	11	10	18
Central venous catheter	2	1		3
Gastrointestinal	2	1		2
Skin/soft tissue	1	1	1	3
Genitourinary tract		1		1
Osteomyelitis			1	
Pneumonia			1	9
Empyema				1
Meningitis				1

tistically significant (P=.086). Many of these additional infections occurred within the first 30 days (11 episodes *v* 3 episodes; P=.235), possibly because of an increase in mucositis in the group given thymic irradiation.

The rate of occurrence of bacterial infections was not influenced by the presence of GVHD. Of the 79 bacterial infections, 40 occurred over 10 287 patient-days of follow-up of patients with GVHD. In comparison, 39 occurred over 11 251 patient-days of follow-up of patients without GVHD (P=.610). Graft rejection was associated with a decrease in the number of bacterial infections, with 66 bacterial infections occurring over 15 845 patient-days of observation in patients with fully donor chimerism and 13 such infections over 5693 patient-days after the grafts were rejected (P=.048).

The rate of bacterial infections was not affected by relapse: 26 bacterial infections after relapse occurred over 6306 patient-days of follow-up, compared with 53 such infections over 15 232 patient-days of follow-up before relapse (P=.478).

Fungal Infections

Seven patients developed *Aspergillus* infections a median of 93 days (range, 40-165 days) posttransplantation; 5 of these patients died a median of 108 days (range, 57-197 days) after transplantation. In 5 cases, *Aspergillus* colonies were isolated from sputum samples, in 1 case from bronchoalveolar lavage fluid, and in another case widespread angioinvasive Aspergillosis was found on postmortem examination. Isolates consisted of *Aspergillus fumigatus* on 5 occasions and *Aspergillus niger* and *Aspergillus versicolor* on 1 occasion each. Five patients had abnormal chest imaging studies consistent with pneumonia at the time sputum cultures were obtained. One patient with fungal sinusitis had a normal chest x-ray but computed tomography scans of the paranasal sinuses demonstrated opacification and air-fluid levels. Two patients were alive at the time of analysis, 398 and 350 days posttransplantation. Five *Aspergillus* infections occurred in patients undergoing treatment for GVHD, 1 patient developed biopsy-proven GVHD 8 days following the isolation of *Aspergillus*, and 1 patient with steroid-resistant acute GVHD was found to have aspergillosis and persistent lymphoma on postmortem examination. Aspergillosis was the most common infectious cause of death in this group of patients.

The only pretransplantation variable associated with the development of *Aspergillus* infection was the use of high-dose ATG for T-cell depletion. The use of 20 or 30 mg/kg ATG was associated with a 6-fold increase in the probability of developing such infections (P=.041). Transplantation from mismatched donors (P=.389), cyclophosphamide dose (P=.374), use of thymic irradiation (P=.433), use of MEDI-507 or ATG for T-cell depletion (P=.288), and choice of DLI strategy (prophylactic, P=.374; therapeutic, P=.304) did not influence the probability of developing *Aspergillus* infection.

The association of *Aspergillus* infection and GVHD failed to achieve statistical significance (P=.136). In 4 cases, GVHD was refractory to steroid therapy and was treated with antithymocyte globulin or anti-CD25 monoclonal antibody therapy or, in 1 case, both agents.

One patient developed symptoms of pneumonia and had an abnormal chest x-ray 5 days after transplantation. *Cryptococcus neoformans* was isolated from the sputum in this case. A leg ulcer discovered on day 8 posttransplantation was caused by infection with *Alternaria* species. This patient is alive and free of disease 1177 days after undergoing HLA-matched stem cell transplantation for chemorefractory non-Hodgkin lymphoma.

Other Viral Infections

Ten viral infections other than CMV were documented. Adenovirus cystitis occurred in 3 patients at a median of 73 days (range, 20-252 days) after transplantation. Infections with herpesviruses occurred on 3 occasions, and included perianal and oral herpes simplex 1 on days 0 and +444, respectively. One patient developed a dermatomal varicella-zoster virus infection on day +259. All herpesvirus infections other than CMV occurred in recipients of matched transplants. Respiratory virus infections were diagnosed in 4 patients at a median of 322 days (range, 12-1064 days) after transplantation. These include 2 parainfluenza and 2 influenza B infections, diagnosed on nasal swab in 2 cases and from sputum and bronchoalveolar lavage fluid in 1 case each. Viral pneumonia was diagnosed only in the case in which parainfluenza was recovered on bronchoscopy.

Protozoan Infections

Two patients developed infections with protozoan organisms. One patient developed fever and right eye pain 402 days after an HLA-matched transplant for non-Hodgkin lymphoma. Pretransplantation serology was equivocal for *Toxoplasma gondii* on 1 occasion and negative on retesting. This patient required treatment with anti-CD25 monoclonal antibody for treatment of GVHD on days 115 and 176 posttransplantation. Retinal examination showed chorioretinitis and retinal detachment. A clinical diagnosis of toxoplasmic chorioretinitis was made and treatment was started with pyrimethamine, clindamycin, and folinic acid. The patient underwent vitreous biopsy but specimens were insufficient to confirm the etiology of the eye pain. The patient failed to recover vision in that eye.

A second patient developed diarrhea 298 days following an HLA-matched transplant for non-Hodgkin lymphoma. Stool examination was positive for *Giardia lamblia* and the patient responded to treatment with metronidazole. This patient never developed GVHD and was never treated with steroids despite receiving 2 donor leukocyte infusions on days 35 and 57 for persistent disease.

DISCUSSION

Despite the hopes of earlier and more complete immune reconstitution following nonmyeloablative allogeneic stem cell transplantation, the recipients in this study had a very high incidence of opportunistic infections. Furthermore, we have documented that the risk of infection persists for an extended period following transplantation, with *Listeria* meningitis diagnosed 427 days after transplantation and CMV infection occurring as late a 483 days posttransplantation. Infections with a broad range of opportunistic pathogens, including CMV, fungal organisms, and infections with low-virulence bacteria such as *Nocardia nova* and *Listeria monocytogenes* were seen. In some cases, these infections may reflect the advanced disease state of our transplantation population because some patients experienced infections with opportunistic pathogens such as *Alternaria*, *Cryptococcus neoformans*, and *Roseomonas* within the first week after transplantation. The range of infections observed in this group of patients is similar to that of recipients of traditional myeloablative bone marrow transplantations.

Cytomegalovirus infection is the most common opportunistic infection following allogeneic stem cell transplantation. Risk factors for reactivation of latent CMV infection include GVHD, treatment with immunosuppressive medications, and donor and recipient serostatus before transplantation. The rate of infection with CMV following myeloablative stem cell transplantation has been reported to be between 38% and 58% [11-14]. Using more sensitive methods of detection, the rate of infection in at-risk patients undergoing myeloablative stem cell transplantation has been reported to be as high as 79% [15]. In a group of 21 patients undergoing nonmyeloablative stem cell transplantation for treatment of hematologic malignancies and solid tumors, the rate of CMV infection was 65% and occurred at a median of 31 days following transplantation. The transplantation preparative regimen in this series included both antithymocyte globulin and fludarabine, and CMV infection preceded the diagnosis of GVHD in each case where both conditions occurred. No statistically significant difference in the rate of infection could be shown between patients with and without GVHD [16].

Recent observations suggest that the choice of nonmyeloablative conditioning regimen may influence the timing and risk factors for CMV infection. Using case-matched control patients, Junghanss et al. [17] showed that while the incidence of a combined end-point of CMV viremia and disease was the same after 1 year for recipients of myeloablative and nonmyeloablative stem cell transplantations, nonmyeloablative transplantation recipients were at much lower risk over the first 100 days. However, more cases of CMV infection were observed late in the first year after nonmyeloablative transplantations. Median time to CMV infection for recipients of nonmyeloablative transplantations who received conditioning with fludarabine and 200 cGy of total body irradiation was 130 days compared with 52 days for recipients of conventional transplants ($P=.02$). The authors hypothesized that residual-host T lymphocytes may contribute to resistance to CMV disease and that, as the levels of these cells decline over the first year following transplantation, the risk of infection increases. In these patients, GVHD was a highly significant risk factor for the development of CMV infection [17]. In contrast, recent observations from 4 British transplantation centers suggest that patients given nonmyeloablative conditioning regimens containing Campath-1H for in vivo T-cell depletion are at risk for early CMV infection. Using a polymerase chain reaction-based assay, the median time to CMV infection reported in this series was only 27 days, with all but 1 initial episode occurring within the first 100 days. No correlation with GVHD was observed in the British cohort [18]. In our series of patients, who were also conditioned with in vivo T-cell depletion, we observed that CMV infection occurred at a median of 53 days posttransplantation, and that all initial episodes of antigenemia occurred within the first 100 days. We have similarly been unable to show a statistically significant correlation between GVHD or steroid use and infection of CMV. The likely explanation for this observation is that extensive T-cell depletion is a strong risk factor for CMV infection, even in the absence of GVHD.

Although our strategy of preemptive therapy for antigenemic patients was highly effective at preventing the emergence of invasive CMV disease, extended treatment with ganciclovir was poorly tolerated, primarily because of myelosuppression. Several patients developed CMV infection on more than 1 occasion, often in conjunction with reduction in the dose of ganciclovir for myelosuppression. In all cases, viremia resolved with reinstitution of full-dose ganciclovir and no nucleoside analogue resistance was documented. Previous work has shown that ganciclovir-induced neutropenia places patients at risk for bacterial infections [12]. Although no relationship between late neutropenia and gram-negative infection could be found in this cohort, late neutropenia remains a significant

cause of concern. The development of antiviral agents active against CMV without the myelosuppressive side effects of ganciclovir and the development of novel methods of preventing and treating CMV infection may result in more effective and better tolerated therapies that prevent the emergence of latent virus infections.

Bacterial infections were a frequent occurrence in our patients. The most common early bacterial infection in the posttransplantation period was bacteremia with coagulase-negative staphylococci, presumably related to the use of indwelling central venous catheters. In a minority of cases, infection was documented on swabs from nonsterile sites. In these cases, colonization with coagulase-negative staphylococci could not be distinguished from actual infection. Despite this potential overestimation, the shift from a predominance of infections with gram-negative organisms to one of gram-positive organisms in neutropenic patients has been well documented [19-21]. In this study, the relative infrequency of gram-negative infections during the first 30 days was likely caused by the prophylactic use of fluoroquinolone antibiotics and the low degree of mucositis that develops with this conditioning regimen. Prophylaxis with fluoroquinolones has been shown to decrease the incidence of gram-negative infection in patients undergoing treatment for acute leukemia, although early mortality is not affected [22,23]. We have observed that graft rejection seemed to protect patients from bacterial infections. It is possible that patients who rejected their grafts were more immunocompetent at the time of transplantation, and were therefore predisposed toward both graft rejection and improved defense against infection. We are currently examining the kinetics of immune reconstitution among patients who undergo bone marrow transplantation using T-cell depleting antibodies to clarify whether this is the case.

Fungal infections were a significant late complication among our patients. In all cases *Aspergillus* infections occurred among patients with GVHD, often in cases where this was refractory to standard immunosuppressive treatment. Five of 7 patients with *Aspergillus* infections have died. Among recipients of myeloablative allogeneic bone marrow transplantations, the rate of invasive fungal infection present at autopsy has been reported to be as high as 40% [24], and in a clinical series *Aspergillus* infection rates were reported to be 15% [25]. Rates of *Aspergillus* infection are known to vary widely with the location of the transplantation center, use of prophylaxis and protective isolation, and the host's degree of immunosuppression. Our results indicate that the risk of late *Aspergillus* infections in patients undergoing nonmyeloablative bone marrow transplantation is similar to that seen in recipients of traditional transplants. *Aspergillus* remained the most common infectious cause of death despite the use of nonmyeloablative conditioning and a relatively short duration of neutropenia. Many patients on this study had early exposure to corticosteroids for treatment of GVHD and engraftment syndrome. These drugs have been shown to increase the rate of growth of *Aspergillus* colonies in vitro and to decrease host resistance to these organisms [26]. The high incidence of invasive *Aspergillus* infections in this cohort suggests a need for improved antifungal prophylaxis with activity against filamentous organisms in recipients of T lymphocyte depleted transplants. There were no cases of invasive disease with *Candida* species, reflecting the use of fluconazole antifungal prophylaxis.

Our results indicate that patients with advanced leukemia and lymphoma who have undergone nonmyeloablative stem cell transplantation remain at significant risk of developing opportunistic infections. The patients in this study were extensively pretreated and had refractory hematologic malignancies at the time of enrollment. At the time of study entry many patients were profoundly immunodeficient, which was indicated by the occurrence of infections with *Alternaria* and *Roseomonas* species within 1 week of transplantation in 2 patients. There was a constant rate of bacterial infection over the first 100 days posttransplantation. Relapse and GVHD did not seem to contribute to the emergence of these bacterial infections. Rates of CMV infection, *Nocardia*, and fungal infections were comparable to those reported by other centers for recipients of myeloablative transplants. Although GVHD may have contributed to the development of fungal infections, no correlation with CMV infection could be found. It is conceivable that in this group of profoundly in vivo T-cell depleted patients a quantitative deficiency of T cells may place patients at significant risk of CMV and other infections, even in the absence of GVHD. T-cell add-back, in the form of DLI, did not seem to reduce the risk of infection, and was associated with an increase in the frequency of CMV infection. Studies currently underway at our institution examining the kinetics of immune reconstitution may help explain these observations. Patients remained at risk for serious infections for an extended period of time after transplantation; *Listeria* and *Nocardia* infections were documented in some patients more than 1 year after treatment. Novel strategies to prevent these infections, including transfer of donor-derived CMV-specific cytotoxic T lymphocytes [27] and other methods of promoting immune reconstitution may improve the tolerability and decrease the rate of serious infections seen among recipients of these transplants.

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